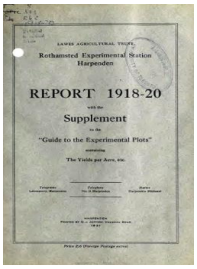
Report 1918-20 With the Supplement to the Guide to the Experimental Plots Containing the Yields per Acre Etc.



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Rothamsted Research

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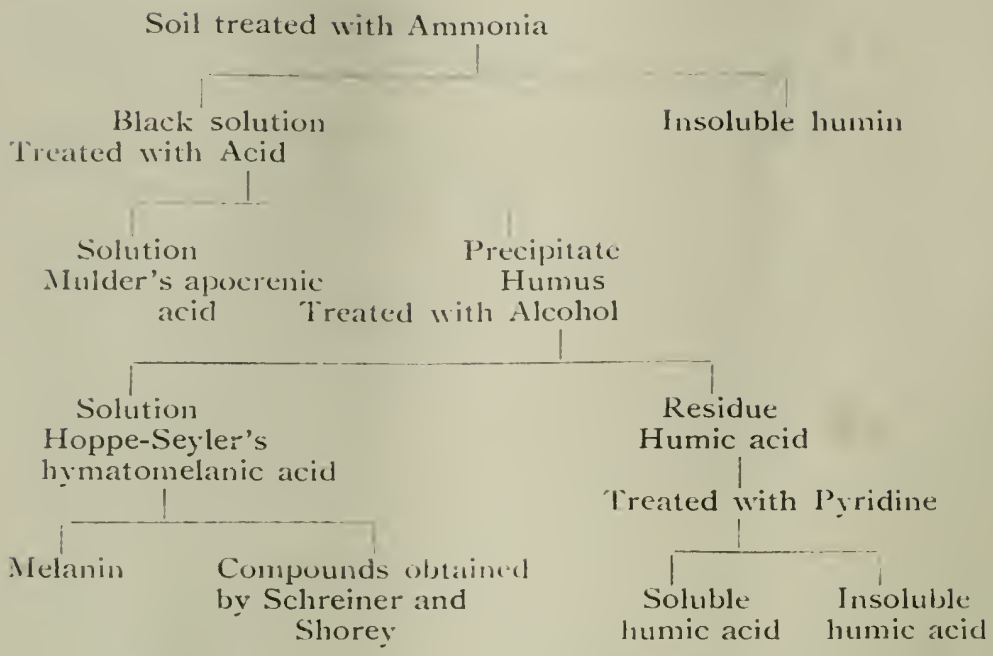

The above procedure has been repeated with rotted straw and with sugar humus, and in both cases similar fractions were obtained. The residue after pyridine extraction of sugar humus was, however, only slowly soluble in ammonia, probably having been converted into humin.

SOIL ORGANISMS.

XIV. L. M. Crump. "*Numbers of Protozoa in certain Rothamsted Soils.*" Journal of Agricultural Science, 1920. Vol. X. pp. 182-198.

The method used was an improvement on that previously adopted in this laboratory, but it did not discriminate between active and encysted forms. Determinations were made at intervals of about seven days of the numbers of total protozoa and bacteria in the soil of Broadbalk Plot 2, which receives 14 tons of farmyard manure in each year, and of Harpenden Field, which is typical of poor arable land. The results are plotted on curves from a study of which the following conclusions are drawn :—

1.—Flagellates, amœbæ and thecamœbæ are usually present in these soils in the trophic condition and in comparatively large numbers, so that there is an extensive population actively in search of food.

2.—The protozoan fauna is practically confined to the top six inches of the soil.

3.—There is a definite inverse relation between the numbers of bacteria and amœbæ.

4.—The amœbæ are uninfluenced by variations in the water content and temperature of the soil and by the rainfall.

5.—The richer the soil is in organic matter the richer it is in protozoa, especially in amœbæ and thecamœbæ.

These conclusions are at variance with those arrived at by the American investigators, but it is believed that the methods employed are better than those used in America.

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XV. D. W. Cutler. *"A Method for Estimating the Number of Active Protozoa in the Soil."* Journal of Agricultural Science, 1920. Vol. X. pp. 135-143.

This method constitutes a great advance on those previously in use, since it discriminates between active and encysted forms; it has, therefore, been adopted in all the succeeding work. The soil is passed through a 3mm. sieve and two samples of 10 grams each are taken. In one the total number of protozoa (active forms plus cysts) is determined as follows: 10 grams of the sieved soil are added to 100 cc. of sterile tap water or physiological salt solution. This gives a 1/10 dilution. From it further dilutions are made as shown below.

No. 1	10 gm. soil	in	100	cc.	H_2O	= 1/10	dilution.
2	10 cc.	No. 1	„	90	„	= 1/100	„
3	5 „	„	2	„	45	„	= 1/1,000	„
4	20 „	„	3	„	30	„	= 1/2,500	„
5	20 „	„	4	„	20	„	= 1/5,000	„
6	30 „	„	5	„	15	„	= 1/7,500	„
7	30 „	„	6	„	10	„	= 1/10,000	„
8	20 „	„	7	„	30	„	= 1/25,000	„
9	20 „	„	8	„	20	„	= 1/50,000	„
10	30 „	„	9	„	15	„	= 1/75,000	„
11	30 „	„	10	„	10	„	= 1/100,000	„

Nutrient agar is poured into sterile Petri dishes. When the medium has solidified, the dishes are inoculated in pairs with 1 cc. of each dilution. Incubation at 20° is continued for 28 days, and the plates examined at intervals of 7 days, 14 days, 21 days and 28 days. This long period of incubation is necessary in order to ensure accurate results.

In the other 10 gram sample the cysts only are determined, advantage being taken of the fact that they survive treatment with 2% hydrochloric acid while active forms do not. The soil is therefore treated with sufficient 2% HCl to neutralise the carbonate present and still leave an excess of unchanged 2% acid. The acid is allowed to act overnight. After treatment, the number of protozoa in the sample is ascertained by the dilution method; this gives the number of cysts since the acid has killed all the active forms, leaving most of the cysts unharmed. The number of cysts subtracted from the total number of organisms given by the first count gives the number of active protozoa per gram of the soil sample.

XVI. D. W. Cutler and L. M. Crump. *"Daily Periodicity in the Numbers of Active Soil Flagellates, with a brief note on the Relation of Trophic Amœbæ and Bacterial Numbers."* Annals of Applied Biology, 1920. Vol. VII. pp. 11-24.

Using the preceding method, it was found that the numbers of protozoa varied so rapidly that weekly counts did not fairly represent the changes taking place. A series of daily counts was there-

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fore projected and continued for 28 days—from February 9th to March 8th. During the last 14 days the bacteria also were counted. The following conclusions were drawn:—

1.—There is a daily variation in the number of trophic forms of the three species of flagellates, *Oicomonas sp.* (Martin), *Cercomonas Longicauda* and *Bodo sp.*, in the soil of arable fields.

2.—The numbers of bacteria and trophic amœbæ in the soil are correlated, varying inversely over a period of 14 days.

3.—Temperature and rainfall appear to have no influence on the number of active protozoa in the soil.

(Note.—In view of the importance of these results counts were begun on July 4th, 1920, and have gone on daily ever since: it is proposed to continue these for 365 consecutive days.)

XVII. D. W. Cutler. "*Observations on Soil Protozoa.*" Journal of Agricultural Science, 1919. Vol. IX. pp. 430-444.

It is shown that soil possesses a remarkable power of retaining protozoa. When a suspension of protozoa is shaken with soil all the organisms are withdrawn until the saturation point is reached, after which, for the first time, the supernatant liquid contains protozoa. Some of the results are:—

	Active flagellates and amœbæ millions per c.c. of suspension			
Before shaking with soil	.56	1.64	1.98	2.80
After „ „ „	Nil.	Nil.	0.29	1.04
Number taken up per gram of soil .	All	All	1.69	1.76

Until the soil has absorbed 1.7 millions per gram there is complete retention of the organisms.

One gram of coarse sand is capable of withdrawing approximately 145,000 amœbæ and flagellates from a suspension of any strength. Fine sand withdraws approximately 980,000: soil and partially sterilised soil 1,650,000, ignited soil 1,500,000, and clay 2,450,000 per gram of material in each case.

These figures are constant for given material and organisms, and are independent of the concentration of the suspensions, the time of action, or whether the suspension contains cysts or active forms of the amœbæ and flagellates investigated. Also the action is the same when the experiment is performed with a suspension of living or dead organisms.

Experiments with the ciliate—*Colpoda cucullus*—show that coarse sand retains 27,000; fine sand 185,000; soil and partially sterilised soil 270,000 and clay 450,000 per gram of material.

The importance of this work arises from the fact that some of the previous investigators have examined soil suspensions under the microscope for protozoa, and have drawn certain conclusions from failure to find active forms. The present investigation shows that the method is unreliable and the conclusions, therefore, not

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justified. This objection does not apply to the dilution method described above.

XVIII. W. F. Bewley and H. B. Hutchinson. *"On the Changes through which the Nodule Organism (Ps. radicicola) passes under Cultural Conditions."* Journal of Agricultural Science, 1920. Vol. X. pp. 144-162.

Under certain cultural conditions the nodule organism from the roots of red clover, broad bean, lucerne and lupin exhibits a tendency towards granular disintegration of the cell with the formation of small non-motile coccoid bodies, about 0.4μ diameter.

In the culture media ordinarily in use these coccoid bodies are not formed extensively, but cultivation on soil extract media rapidly leads to their production, until finally they constitute the predominant type in the culture.

A life-cycle consisting of five stages is described :—

1.—*The pre-swarmer form (non-motile).* When a culture of the organism is placed in a neutral soil solution, it is converted after four or five days into the pre-swarmer form.

2.—*Second stage, larger non-motile coccus.* In presence of saccharose, certain other carbohydrates and phosphates, etc., the pre-swarmers undergo a change. The original coccoid pre-swarmer increases in size until its diameter has been doubled, but still remains a non-motile coccus.

3.—*Swarmer stage, motile.* The cell then becomes ellipsoidal and develops high motility. This form is the well-known " swarmer " of Beijerinck.

4.—*Rod-form.* Proceeding in an " up-grade " direction, the swarmer becomes elongated and gives rise to a rod-form, which is still motile but decreasingly so. So long as there is sufficient available carbohydrate in the medium, the organism remains in this form.

5.—*Vacuolated stage.* When, however, the organism is placed in a neutral soil extract or the available carbohydrate becomes exhausted, it becomes highly vacuolated and the chromatin divides into a number of bands. Finally, these bands become rounded off and escape from the rod as the coccoid pre-swarmer.

The formation of the coccoid bodies (pre-swarmers) may also be induced by the addition of calcium or magnesium carbonates to the medium or by placing the organisms under anærobic conditions. Of a considerable number of compounds other than carbohydrates, calcium phosphate alone was capable of bringing about the change from pre-swarmers to rods.

The organism also appears to be affected greatly by the reaction of the soil. In the main, the normal rod rapidly changes into the pre-swarmer form in calcareous soils ; acid soils cause the production of highly vacuolated cells and eventually kill the organism, while a slightly alkaline soil was found to be capable of supporting vigorous growth without altering the form of the cells.

The effect of various temperatures on the rapidity of pre-swarmer formation has been studied. Relatively high temperatures (30° and 37°) either prevent or postpone the entrance of down-grade changes.

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XIX. H. B. Hutchinson and J. Clayton. "*On the Decomposition of Cellulose by an Aerobic Organism (Spirochæta cytophaga n. sp.).*" Journal of Agricultural Science, 1919. Vol. IX. pp. 143-173.

Examination of Rothamsted soils on different occasions has revealed the presence of an organism capable of breaking down cellulose with comparative ease. Morphologically, the organism appears to possess greater affinities with the Spirochætoideæ than with the bacteria, and the name *Spirochæta cytophaga* is therefore suggested.

While the Spirochæt is capable of considerable vegetative growth as a sinuous filamentous cell, it also appears to pass through a number of phases which terminate in the production of a spherical body (sporoid) which differs in a number of respects from the true spores of the bacteria. Germination of the sporoid again gives rise to the filamentous form, which possesses perfect flexibility and is feebly motile. The latter does not apparently possess flagella.

Spirochæta cytophaga is essentially aerobic; its optimum temperature is in the region of 30°. Both the thread and sporoid stages are killed by exposure to a temperature of 60° for ten minutes.

The nitrogen requirements of the organism may be met by a number of the simpler nitrogen compounds—ammonium salts, nitrates, amides and amino-acids. Peptone is also suitable in concentrations up to 0.025%. Stronger solutions, *e.g.*, 0.25% lead to a marked inhibition of growth. The organism fails to grow on the conventional nutrient gelatine or agar.

Comparative experiments with a number of higher alcohols, sugars and salts of organic acids show that none of these is capable of meeting the carbon requirements of the organism. Cellulose is the only carbon compound with which growth has been secured.

Although none of the monoses, bioses and other carbohydrates is able to support growth, many of them exert an inhibitive action on cellulose decomposition if present in other than very low concentrations. This may be correlated with the reducing properties of the carbohydrate. Maltose, for example, has been found to be approximately 70 times more toxic than saccharose.

Of the various by-products of the action of Spirochæta cytophaga may be mentioned: (*a*) a pigment possessing relations to the carotin group, (*b*) mucilage which does not give rise to optically active compounds on hydrolysis, and (*c*) small quantities of volatile acids.

Evidence is also adduced to show the relation of cellulose decomposition to the assimilation of atmospheric nitrogen.

XX. A. W. Rymer Roberts. "*On the Life History of Wireworms of the genus* Agriotes, *Esch., with some Notes on that of* Athous Hæmorrhoidalis, F." Part I. Annals of Applied Biology, 1919. Vol. VI. pp. 116-135.

The biology and life history of the common "wireworm" was studied during the years 1916-1919. In England and probably also in Wales and Scotland, *Agriotes obscurus* is generally the

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commonest species. The adult beetles hatch from the pupa in August or September and remain in hibernation during the winter. About the middle of May they emerge, feed on the nectar or pollen of flowers and do little or no damage, at least in this country. Oviposition takes place generally from the end of June to the middle of July. The eggs of three species of *Agriotes*—*obscurus*, *sputator* and *sobrinus* and *Athous hæmorrhoidalis* were obtained from the soil of pots, in which the beetles had been confined, at depths varying from $\frac{1}{4}$-inch to 2 inches, either in batches or singly. Attempts to obtain ova from *Ag. lineatus* failed, but from other sources it is known to deposit its eggs in a similar position, and probably the presence of grasses, whether cultivated or growing as weeds, is essential to all five species. This conclusion points to the necessity for clean cultivation in the control of wireworms.

The larvæ on emergence at once burrow into the soil. All are pale in colour and so small (1-2.75mm.) as not to be generally recognised during their first year. The first moult of *A. obscurus* takes place in June, the second in July or August, and it is believed that the larvæ in general moult twice a year, in April or May, and again between July and September. In their first year, the larvæ appear to feed chiefly on partially decomposed vegetable matter and perhaps to some extent on the small roots of living plants, but no evidence of definite damage was obtained. In the later stages they feed on almost any crop and on many weeds. They appear to attack mustard only in the absence of more suitable food, though they are frequently found at the roots of charlock. The larvæ can subsist for a long time on the decaying organic matter in the soil and are able to withstand immersion in water for prolonged periods. During the winter they may be found close to the surface in grass land. But in fallow land they undergo a period of hibernation, sometimes as much as 2ft. from the surface.

Agriotes obscurus has a larval life history extending to five years, as was originally stated by Bierkander.

Pupation takes place in an earthen cell prepared by the larvæ at a depth of from 1 inch up to $7\frac{1}{2}$ or more inches. The pupal stage extends over a period of about 3 weeks, pupæ being found from the end of July to the middle of September.

Wireworms under natural conditions are not parasitized to any great extent. A Proctotrupid, probably *Phænoserphus fuscipes* Hal. was bred from *Athous hæmorrhoidalis*, and a Proctotrupid was also found within a larval *Agriotes obscurus*. The latter species was also found to be the host of a fungus of the genus *Isaria*.

XXI. F. Tattersfield and A. W. R. Roberts. "*The Influence of Chemical Constitution on the Toxicity of Organic Compounds to Wireworms.*" Journal of Agricultural Science, 1920. Vol. X. pp. 199-232.

The relationship between chemical constitution and toxicity to wireworms of organic compounds is found to be of a two-fold nature.

The general effect of a group of compounds of the same type is directly determined by the chemical constitution of the type.

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The particular effects of individual members of the groups are limited by their physical properties such as volatility, etc., which may be regarded as indirect consequences of their chemical constitution.

The aromatic hydrocarbons and halides are on the whole more toxic than the aliphatic hydrocarbons and halides. The groups that influence toxicity most when introduced singly into the benzene ring are in order of importance the methylamino (most effective), dimethylamino, hydroxy, nitro, amino, iodine, bromine, chlorine, methyl groups (least effective). But this order is modified in presence of another group; thus when there is a CH_3 already present in the ring the order becomes chlorine (side chain), amino, hydroxy, chlorine (ring), methyl. Chlorine and hydroxy groups together give rise to highly poisonous substances considerably more effective than where present separately. The association of chlorine and nitrogroups in chlorpicrin give rise to one of the most toxic substances tested. Methyl groups substituted in the amino group of aniline increase toxicity more than if substituted in the ring.

Compounds with irritating vapours have usually high toxic values, *e.g.*, Allyl isothiocyanate, chlorpicrin, benzyl chloride. The toxic values of these substances are not closely correlated with their vapour pressures or rates of evaporation.

There is a fairly close relationship between toxicities and the vapour pressure, rates of evaporation and volatilities of compounds of the same chemical type. In a series of similar compounds decreases in vapour pressure and volatility are associated with an increased toxicity. A possible explanation is that condensation or absorption takes place along the tracheal system when insects are submitted to the action of these vapours. On exposure once more to the open air these vapours diffuse out into the atmosphere, the rate at which they do so being a measure of the rapidity with which the insect recovers.

A limit is put upon toxicity by the decrease in vapour pressure, when it sinks too low to allow a toxic concentration in the vapour phase. Chemically inert compounds boiling above 170° C. are generally uncertain in their poisonous effects on wireworms after an exposure of 1,000 minutes at 15° C. Nearly all organic compounds boiling above 215° C. are uncertain in their action, while those boiling above 245° C. are non-toxic. These limits depend on the resistance of the insect, the length of exposure and the temperature at which the experiment is carried out.

XXII. N. N. Sen Gupta. "*Dephenolisation in Soil.*" Journal of Agricultural Science, 1921. Vol. XI.

It is found that phenol and the cresols disappear when added to soil. Three actions seem to be involved:—

1.—An instantaneous disappearance which appears to be non-biological, but its exact nature has not yet been elucidated; apparently it varies directly with the clay content of the soil.

2.—A slow decomposition which continues till all the phenol is exhausted. This is apparently largely brought about by micro-organisms capable of utilising phenol as a source of energy.

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3.—There appears, however, to be some non-biological slow decomposition also, since the decomposition in unmanured soil poor in micro-organisms is much slower than in manured soils, and altogether different in character.

Autoclaving the soil at 130° for 20 minutes destroys the cause or causes of the decomposition altogether, but the action proceeds, although much more slowly, than in untreated soil, in the presence of a considerable amount of toluene and mercuric chloride.

Partial sterilisation by treatment with toluene which was evaporated before the addition of phenol increases the rate of decomposition, but steaming does not.

The decomposition takes place even in soil air-dried to 2.4% moisture, but it is extremely slow compared with the rate in normal soil.

When successive doses of phenol are applied to the same soil, each dose is decomposed at a higher rate than the preceding one. This is entirely in accordance with a decomposition mainly biological in character. The same effect has been observed in the case of *m*-cresol.

The treatment of the soil with sulphuric acid (50% by volume) either before or after the addition of phenol greatly augments the instantaneous loss, which may amount to 90% in case of phenol This loss is not affected by autoclaving.

CONDITIONS DETERMINING ENVIRONMENTAL FACTORS IN THE SOIL.

XXIII. B. A. Keen. "*A Note on the Capillary Rise of Water in Soils.*" Journal of Agricultural Science, 1919. Vol. IX. pp. 396-399.

A simple formula for the theoretical maximum rise in an ideal soil, composed of closely packed and uniform spherical grains, may be obtained from a consideration of the triangular pores existing in such a soil. The formula reduces to $h=\frac{.75}{r}$ where h = height of rise and r = radius of spherical grain. The capillary rises given in the following table are calculated on the assumption that a soil is made up entirely of one given soil fraction, and not of a mixture of fractions, and the particles are taken as closely packed spheres :—

Soil Fraction	Diameter in mm.		Capillary Rise in cms.		Average Rise in Ft.
	Max.	Min.	Min.	Max.	
Fine gravel .	3	1	5	15	$\frac{1}{3}$
Coarse sand .	1	.2	15	75	$1\frac{1}{2}$
Fine sand .	.200	.040	75	375	$7\frac{1}{2}$
Silt . . .	.040	.010	375	1500	$31\frac{1}{4}$
Fine silt . .	.010	.002	1500	7500	150
Clay . .	.002	—	7500	—	150 upwards

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